(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 189 673**
A1

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 85309462.1

(22) Date of filing: 23.12.85

(51) Int. Cl.⁴: A 61 K 37/43
A 61 K 37/02

(30) Priority: 24.12.84 JP 280036/84
18.09.85 JP 207244/85

(43) Date of publication of application:
06.08.86 Bulletin 86/32

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI NL SE

(71) Applicant: SUMITOMO PHARMACEUTICALS COMPANY, LIMITED
40, Dosho-machi 2-chome
Higashi-ku Osaka-shi Osaka-fu(JP)

(72) Inventor: Fujioka, Keiji 206 Famille Tsukaguchi
27-5, Tsukaguchicho 1-chome Amagasaki-shi
Hyogo 661(JP)

(72) Inventor: Sato, Shigeji
2-29-7, Ooike Ibaraki-shi
Osaka 567(JP)

(72) Inventor: Takada, Yoshihiro
18-1-1-404, Tamagawa 1-chome
Takatsuki-shi Osaka 569(JP)

(74) Representative: Sheard, Andrew Gregory et al,
Kilburn & Strode 30, John Street
London WC1N 2DD(GB)

(54) **Stable growth hormone releasing factor preparation.**

(57) A growth hormone releasing factor (GRF) preparation, which has good stability, has human serum albumin or glycine incorporated in it, with or without buffer. The amount of human serum albumin or glycine contained is usually 100 mcg to 30 mg per 100 mcg GRF. The preparation may be in solid or solution form, and is preferably lyophilized. The amount of buffer, if added, is preferably enough so that pH of the preparation is kept at from 2 to 7. More preferably, the preparation is kept in a nitrogen gas atmosphere. The lyophilized preparation is readily usable for, for example, injection.

EP 0 189 673 A1

STABLE GROWTH HORMONE RELEASING FACTOR PREPARATION

This invention relates to a stable growth hormone releasing factor (hereinbelow abbreviated to GRF) preparation; more precisely, it relates to a GRF preparation containing human serum albumin or glycine as a stabilizer.

GRF is peptide purified and isolated by Guillemin et al from the pancreas of a patient with pancreatic tumour showing symptoms of acromegaly. It has the primary structure of amino acids as follows: H-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu -Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met- Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu $-NH_2$ (Guillemin, R., Brazeau, P., Poehlen, P., Esch, F., Ling, N., & Wehrenberg, W. B.: "Growth Hormone Releasing Factor from a Human Pancreatic Tumor that Caused Acromegaly"; Science, 218, 585-587, 1982). It is later identified that it has exactly the same structure as GRF purified from the hypothalamus, and the name "somatocrinin" in place of GRF has been offered.

GRF has so far been isolated and purified as peptides composed of 44, 40, 37 and 29 amino acids, and all of them have been confirmed to have growth hormone releasing activity. These peptides are expected to be administered temporarily or for a long period for diagnosis or medical treatment in clinical medicine (both human medicine and veterinary medicine).

However, GRF is rather unstable and hardly preservable in solution. It is desirable for a pharmaceutical preparation to keep GRF in the form of a lyophilized

preparation. But the lyophilized preparation is still not satisfactory, showing a significant reduction in titre when preserved at room temperature over a long period or exposed to heating, humidity or light. One of the causes is that methionine at position 27 of amino acids forming GRF is liable to be oxidized. Although attempts had been made to stabilize it by addition of antioxidants such as L-ascorbic acid, no lyophilized preparations with enough long-term stability have been obtained yet.

After strenuous efforts to resolve the difficulty, the present inventors have found that human serum albumin or glycine greatly facilitates an improvement in stability of GRF and a preparation of practical use is obtained. This invention is based on this finding.

According to a first aspect of the invention there is provided a growth hormone releasing factor (GRF) preparation comprising GRF, the preparation being characterized in that it comprises human serum albumin or glycine.

According to a second aspect of the invention there is provided a method of stabilizing growth hormone releasing factor (GRF), the method being characterized by adding human serum albumin or glycine (and in either case optionally a buffer) to the GRF.

According to a third aspect of the invention there is provided a growth hormone releasing factor (GRF) preparation comprising GRF, the preparation being characterized in that it comprises human serum albumin or glycine, for use in human or veterinary medicine.

According to a fourth aspect of the invention there is provided the use of a growth hormone releasing factor (GRF) preparation comprising GRF, the preparation being characterized in that it comprises human serum albumin or glycine, in the preparation of a pharmaceutical or veterinary agent.

Any GRF may be used in the present preparation as long as it is a peptide having growth hormone releasing activity. For example, it does not matter whether the number of amino acids is 44, 40, 37 or 29. The GRF may comprise a mixture thereof.

There is no limitation to the dosage form of the present GRF preparation. That is, it may be a solution or a solid preparation. A lyophilized preparation is preferable as a pharmaceutical preparation with long-term stability. It is more preferable that nitrogen gas be introduced into an ampoule of the lyophilized preparation. The lyophilized preparation of GRF concerned with this invention has a remarkable stability compared to preparations containing other additives. The preferred lyophilized preparation is also able to be freely dissolved at the time of use by addition of some appropriate dissolving solution such as distilled water for injection or physiological saline solution. Conveniently, this dissolution may be conducted just before use for injection.

There is no critical limitation to the amount of human serum albumin or glycine to be added, but it will be appropriate to add from 100 mcg to 30 mg per 100 mcg GRF.

In order to make the pr paration mor stable, it is

preferred to add buffer. Any buffer which is appropriate for medical preparations may be used, for instance, acetate, phosphate or citrate. In the case of a liquid preparation such as an injectable preparation or an intranasal drug, the amount of the buffer to be added may usually be enough to keep the preparation's pH at 2 to 7, preferably 2.5 to 5, taking the physical and chemical stability of GRF into consideration. In the case of a lyophilized preparation, the amount of addition of the buffer may be such that the pH of the preparation is kept within the same range as above, after re-dissolution. The present preparation may further contain additives such as an isotonizer, a soothing agent and an excipient.

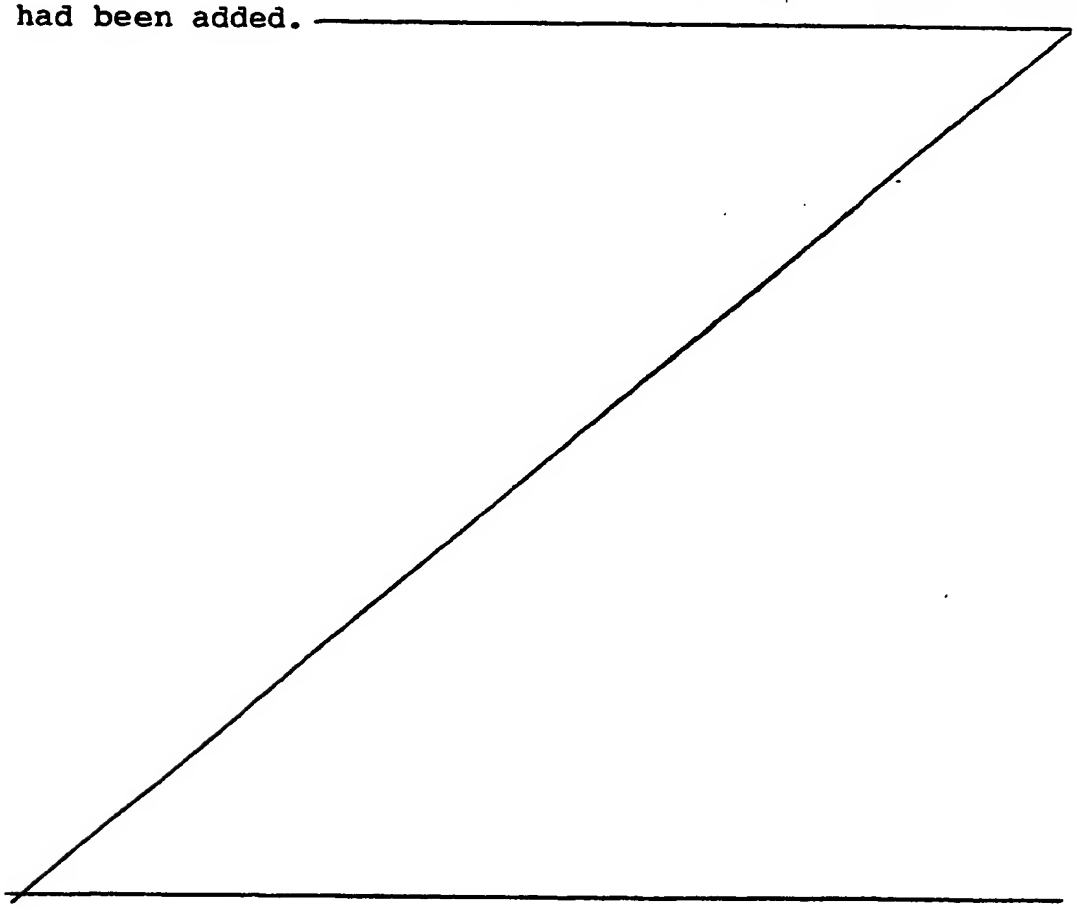
The present preparation may be prepared by any appropriate method; they will be familiar to those skilled in the art. For example, in the case of an injectable preparation, human serum albumin or glycine is simply added to distilled water for injection or to an appropriate buffer, then GRF is added to it until it is dissolved and then the solution is subjected to filtration for sterilization. The solution thus obtained may then be lyophilized in a conventional manner. If necessary, nitrogen gas is introduced into an ampoule to obtain an injection preparation lyophilized and kept under nitrogen.

It will be understood that the present invention includes other forms of sterile preparations.

This invention is illustrated by the following experiments and examples.

Experiment 1

An experiment was conducted in order to confirm the stabilizing effect of this invention. Various stabilizers were dissolved in aqueous solutions containing GRF (1-44) and lyophilized, and then nitrogen gas was introduced. Then, examination was made for the qualities listed in Table 1. It was found that the lyophilized preparation to which human serum albumin or glycine had been added showed a remarkable stability compared to those to which other stabilizers had been added.



Exp riment 2

An experiment was conducted in order to confirm the stabilizing effect of this invention. Various stabilizers were dissolved in phosphate buffer solutions containing GRF (1-29). These solutions were lyophilized and nitrogen gas was introduced. Then examination was made for the items listed in Table 2. It was found that the stability of GRF (1-29) was increased by the addition of human serum albumin or glycine.

On the other hand, the lyophilized preparations added cysteine or sodium thiosulphate showed a decrease of potency and changed in appearance.

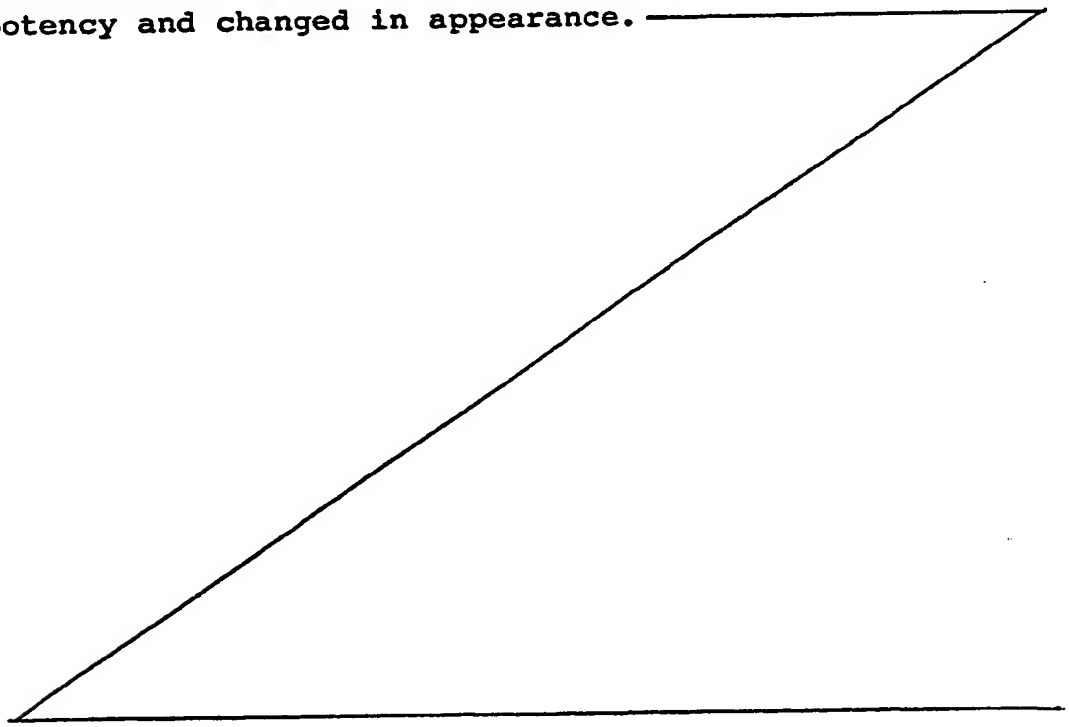


Table 1: Comparison of stabilizers [GRF(1-44)]

Storage conditions	Stabilizer / Item	Human serum albumin (1 mg)	Glycine (4 mg)	D-mannitol (10 mg)	L-ascorbic acid (1 mg)
Initial	Description of solution	Colourless & clear	Colourless & clear	Colourless & clear	Colourless & clear
	Content (%)	100	100	100	100
50°C for 15 days	Description of solution	Colourless & clear	Colourless & clear	Colourless & clear	Pale Yellow
	Content (%)	99	81	52	42

Note: Figures in parentheses indicate the amount of stabilizer added per 100 µg GRF (1-44). GRF content was assayed by high performance liquid chromatography.

Table 2: Comparison of stabilizers [GRF(1-29)]

Storage conditions	Item \ Stabilizer	No addition	Human serum albumin (1 mg)	Glycine (10 mg)	Human serum albumin (1 mg) Cysteine (2 mg)	Human serum albumin (1 mg) Sodium thiosulfate (2 mg)
Initial	Description of solution	Colourless & clear	Colourless & clear	Colourless & clear	Colourless & clear	Colourless & clear
	pH	5.3	5.4	5.3	2.8	5.0
	Content (%)	100	100	100	100	100
50°C for 15 days	Description of solution	Colourless & clear	Colourless & clear	Colourless & clear	Colourless & clear	Opalescence
	Content (%)	65	95	89	22	13

Note: Figures in parentheses indicate the amount of stabilizer added per 100 µg GRF (1-29). GRF content was assayed by high performance liquid chromatography.

0186673

Some examples are now given. The invention is not limited to them.

Example 1

200 mg of human serum albumin was added to 10 ml of acetic acid-sodium acetate buffer at pH 5, and then 10 mg of GRF (1-44) was dissolved. After this solution was filtered through a sterile filter and dispensed as 100 mcl of solution per 4 ml glass vial and lyophilized. After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 2

100 mg of human serum albumin was added to 50 ml of phosphoric-citric buffer at pH 3, and then 10 mg of GRF (1-44) was dissolved. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized. After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 3

400 mg of glycine was dissolved in 50 ml of distilled water for injection, and then 10 mg of GRF (1-44) was dissolved. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized. After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 4

4 mg of GRF (1-29) was dissolved in 20 ml of 1/10 M

phosphate buffer at pH 5, and then 40 mg of human serum albumin was added. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized.

After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 5

4 mg of GRF (1-29) was dissolved in 20 ml of 1/10 M phosphate buffer at pH 5, and then 40 mg of human serum albumin and 280 mg of sodium chloride were added. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized.

After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 6

4 mg of GRF (1-29) was dissolved in 20 ml of 1/10 M phosphate buffer at pH 5, and then 40 mg of human serum albumin and 360 mg of sodium chloride were added. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized.

After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 7

4 mg of GRF (1-29) was dissolved in 20 ml of 1/10 M

phosphate buffer at pH 5, and then 40 mg of human serum albumin and 400 mg of mannitol were added. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized.

After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 8

20 mg of GRF (1-29) was dissolved in 20 ml of 1/10 M phosphate buffer at pH 5, and then 40 mg of human serum albumin was added. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized.

After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 9

20 mg of GRF (1-29) was dissolved in 20 ml of 1/10 M phosphate buffer at pH 5, and then 200 mg of human serum albumin and 280 mg of sodium chloride were added. After this solution was filtered through a sterile filter and dispensed as 500 mcl of solution per 4 ml glass vial and lyophilized.

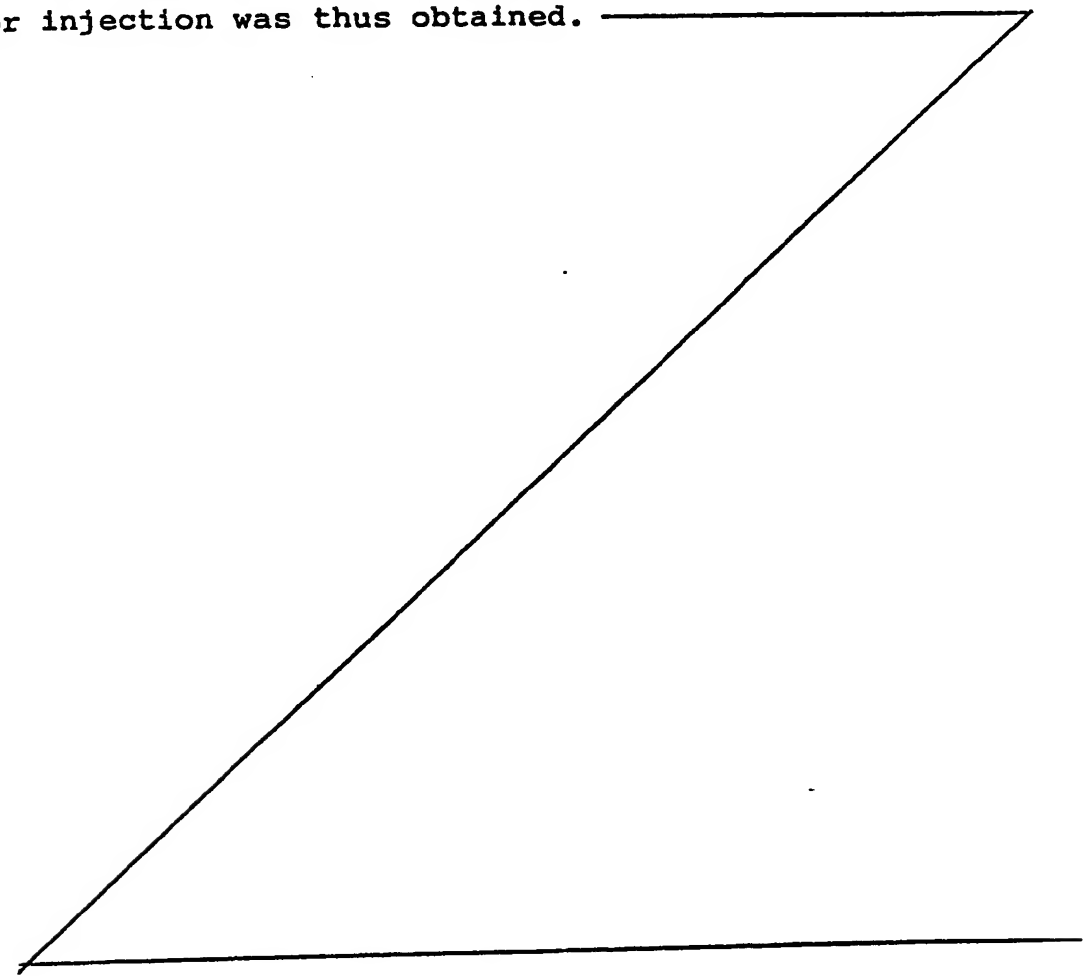
After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.

Example 10

20 mg of GRF (1-29) was dissolved in 100 ml of 1/10 M

phosphate buffer at pH 5, and th n 200 mg of human serum albumin and 1.4 g of sodium chloride were added. After this solution was filtered through a sterile filter and dispensed as 2.5 ml of solution per 4 ml glass vial and lyophilized.

After lyophilization, the vial was closed under a nitrogen atmosphere. A stable lyophilized preparation for injection was thus obtained.



CLAIMS

1. A growth hormone releasing factor (GRF) preparation comprising GRF, the preparation being characterized in that it comprises human serum albumin or glycine.

2. A preparation as claimed in Claim 1, characterized in that it contains buffer.

3. A preparation as claimed in Claim 2, characterized in that the buffer is of such a nature and present in such an amount that the pH of the preparation is from 2 to 7.

4. A preparation as claimed in Claim 1 or 2 characterized in that it is lyophilized.

5. A preparation as claimed in Claims 2 and 4, characterized in that the buffer is of such a nature and present in such an amount that the pH of a solution of the lyophilized preparation has a pH of from 2 to 7.

6. A preparation as claimed in any one of Claims 1 to 5, characterized in that it is kept in an atmosphere of nitrogen gas.

7. A method of stabilizing growth hormone releasing factor (GRF) the method being characterized by adding human serum albumin or glycine (and in either case optionally a buffer) to the GRF.

8. A method as claimed in Claim 7, characterized by lyophilizing the preparation so formed.

9. A method as claimed in Claim 7 or 8, characterized in that the buffer, when present, is of such a nature and present in such an amount that the pH of the preparation so formed, or in the case of a lyophilized preparation the pH of a solution of the lyophilized preparation so formed, is from 2 to 7.

10. A method as claimed in Claim 7, 8 or 9, characterized in that the preparation so formed is kept in an atmosphere of nitrogen gas.

European Patent Office

EUROPEAN SEARCH REPORT

0189673
Application number

EP 85 30 9462

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
A	US-A-3 803 309 (PIERRE ANTOINE DESAULLES)	1-10	A 61 K 37/43 A 61 K 37/02

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 61 K
C 07 C

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	07-04-1986	BRINKMANN C.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO Form 1503 03.82